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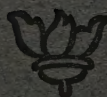
BY

MARY K. BRYAN

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*Reprinted from* **JOURNAL OF AGRICULTURAL RESEARCH**  
Vol. XXI, No. 3 : : : : - Washington, D. C., May 2, 1921

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**PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH  
THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES**

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WASHINGTON : GOVERNMENT PRINTING OFFICE : 1921

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## A BACTERIAL BUDROT OF CANNAS

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### INTRODUCTION

The disease described in this paper was first observed by the writer in July, 1918, when the unsightly appearance of hybrid canna plants (*Canna indica* L.) in the public grounds in Washington (Pl. 31) drew attention to it. The most noticeable lesions at that time were large, irregular brown spots which caused distortions of the leaves. Close inspection showed that many plants were attacked to a greater or less degree; leaves of all ages were involved, and often young shoots were killed by the destruction of the bud. A microscopic examination of sections of diseased areas disclosed the presence of swarms of bacteria in the tissues; and, as no mention of the disease was found in literature, a definite investigation was undertaken.

### APPEARANCE OF DISEASED PLANTS

This disease is essentially one of young tissues and moist conditions. This is evident from the virulence of the attack on buds as well as from the fact that infections make little headway on mature leaves.

The spots on the leaves vary in size from minute stomatal infections to ragged, brown, irregular areas extending several inches along the blade, usually between midrib and margin (Pl. 32, 33). Small stomatal infections are found in great numbers near the margins of large spots or on leaves with no large lesions, and on mature leaves they do not develop further. On young leaves they enlarge into spots which tend to run between the parallel veins, giving to the leaf a striped effect. This effect is continued in the uneven margins of large spots formed by the coalescing of small ones, where often a diseased strip between two veins runs an inch or more beyond the main diseased area into otherwise healthy tissue. Young infections and the advancing edges of larger spots are first water-soaked, then yellow, later becoming brown. In the early morning water-soaked streaks extend far beyond the yellow tissue. Old spots are dry, thin, and grey-brown, and by shrinking cause distortions of the leaves (Pl. 32, 33). They show a dark, almost black, mottling or checkering, which is more plainly seen by transmitted than by reflected light, the



different colored areas being rectangular rather than round. This mottled appearance and the position of the spots between midrib and margin clearly distinguish this disease from the common dying back of the older leaves, where the margin is first to succumb and the dead areas are a uniform red-brown.

Infection usually takes place while the leaves are still rolled in the bud. In case stomatal infections occur just before the leaf pushes up, the whole bud often looks very pale, almost white, and close examination shows it to be covered with minute white spots. Sometimes these do not spread further, and the leaf as it matures becomes green with a peppering of tiny spots, but more often as it unfolds it remains pale and stunted. At other times the infection has progressed so far that by the time the folded leaf emerges it is wholly or in part blackened, sometimes in spiral bands (Pl. 35, B). In such cases the younger leaves usually become infected by direct contact, or the disease runs down the petiole and kills the young stalk and bud. A non-fatal bud infection is shown in Plate 35, A, where blackening has occurred but not to such an extent as to kill the shoot.

From leaf-blade infections (Pl. 33; 36, C) the bacteria invade the petiole, not by way of the vascular system but through the parenchyma, chiefly through the channels of very loose tissue which occupy a large portion of the interior of the petiole. In the tightly rolled buds infection appears to pass directly from one leaf to another, so that when a young shoot is cut across near ground level, several petioles may be found to be diseased. The buds do not become soft-rotted but usually stand up black and dry or are bent over or broken off. Eventually the center rots out, leaving the hollow stalk standing with one or two mature leaves. This when cut across near the base is found to contain a watery rot. The rootstocks have never been found to be diseased. In the autumn of 1919 when the plants in the grounds were lifted for winter storage all the plants of one large bed that had shown heavy infection were thoroughly examined. All stalks were cut across a few inches from the ground, and in a large proportion of the clumps one to four stalks were found with interiors rotted out, to or below ground level, the lower part of the cavity being filled with fluid. In no case, however, was the rot found extending into the rootstock, the tissues of which do not seem to favor the growth of the organism. In many stalks showing a characteristic top—that is, standing erect with older leaves intact but with a hollow blackened center, the decay had not reached the lower part, so that a cross section 1 foot from ground level showed only sound tissue.

Often when the shoot has escaped early and complete destruction, the flower clusters are ruined either by the infection of the young flower buds or by the decay of the stem. In the former instance the stem and pedicels develop but the buds blacken and die while still rudimentary (Pl. 34, A, C). In the latter case the stalk bends or breaks in the infected



region (Pl. 34, B). Infection often remains on one side of the stalk, which blackens and, if infected very young, fails to elongate like the healthy side and so cracks across at frequent intervals, the cracks becoming gummy with the exuded sap (Pl. 36, C). Sometimes the rot extends along the stalk to its tip, blackening pedicels and well-formed buds.

#### SUSCEPTIBLE VARIETIES

During the summer of 1918 most of the varieties observed were healthy or showed only a trace of this disease. The badly affected sorts were Princeton, Gayety, City of Portland, and Charles Lutz. The disease was most virulent in the early summer, many plants recovering during August.

In 1919 the outbreak was much more virulent than in the preceding year, but the susceptible varieties were different. This time the Yellow King Humbert, a sport from the Red King Humbert, and Carmine Beauty were most injured. Many of the varieties planted in 1918 were not set out in 1919, so no comparison could be made. It was learned from one of the gardeners that some varieties had been dropped in the past because of this budrot, among them Fire Bird and Mrs. Alfred Conard. Another gardener ascribed all the trouble to overwatering and crowding in the hothouse before setting-out time, conditions undoubtedly very favorable to the activities of the causal organism.

#### DAMAGE DONE

In 1919 the disease was observed earlier than in the previous year—that is, in the latter part of May soon after the plants were set out. At this time there were only scattering infections, a few large leaf spots, and several infected and dead shoots. Later (June 19) several beds showed from 10 per cent to 80 per cent of infected plants; of these many had two or three of the four shoots involved, and eight plants in one bed had bent blossom stalks.

During July many plants outgrew the disease by sending out new, vigorous shoots; but in August, although to the casual observer no traces of disease were present, a great many unsightly leaves and some sickly young shoots might be found, and often a blossom stalk pushed up through a ragged brown sheath.

In May, 1920, potted plants in the hothouse ready for setting out were examined, and the following varieties were found severely infected—that is, with a scattering of dead or diseased buds: Yellow King Humbert, Gayety, Golden Eagle, Dazzler, Favorite, and Wallace.

Other varieties on the same bed and subject to the same conditions were entirely free from signs of the disease. These were Meteor, Olympic, Rosea Gigantea, Fenal, President, Princeton, and City of Portland.

No connection could be traced between infected beds of one year and the serious attack of the following year. The beds most heavily infected in 1918 were in some cases almost disease-free in 1919, others were badly



infected, while some beds where no disease occurred in 1918 showed the highest percentage of infection in 1919. These observations, however, are complicated by the fact that different varieties, the susceptibility of which is not known, were planted the second year. From present knowledge it seems that the disease must be carried over on the rootstocks, especially since the trouble begins to develop before the plants are taken from the hothouse.

Overwatering of the foliage appears to be a large factor in the development of the disease, since rootstocks which were taken from heavily infected clumps and kept under favorable conditions during the winter and carefully watered when potted in early spring gave not a single case during the entire summer, while rootstocks from the same source without special care showed a large percentage of infection before setting-out time.

#### GEOGRAPHICAL DISTRIBUTION

The disease has thus far been observed only in the District of Columbia and in Illinois. Typically infected plants were found at Urbana, Ill., in the summer of 1920.

#### ISOLATIONS

When sections were made of diseased tissues, motile bacteria were invariably found in great numbers swarming out on the slide. Plates were poured on peptone-beef agar from leaf spots, pedicels, and from petioles near the ground level. In every case practically pure cultures of a white bacterial organism were obtained. Judged from plate colonies, the organism was the same in every case, and comparative cultural studies of several isolations have corroborated this judgment.

#### INOCULATIONS

Inoculations were made in 1918 on potted cannas in the hothouse. These plants were not in good condition but were the only ones available at the time. Young leaves just unfolded were inoculated by placing drops from a young agar slant culture on the surface and making delicate pricks in the blade through these. Drops were also poured into the tips of tightly rolled leaves without wounding. Subcultures of isolations from leaf blades and from petioles were used. Some plants were kept moist for 36 hours by spraying in cages with sterile water; others were left in the open hothouse.

In most cases no infections appeared. One inoculated leaf showed on the fourth day several water-soaked streaks 1 to 10 mm. long running from needle pricks. These turned yellow then brown, but did not spread further. Plates were poured from the edge of the longest streak, and colonies were obtained which appeared to be right. Transfers from these conformed to the original isolation in subsequent cultural tests.

Attempts were made to infect the slow-growing cannas with the re-isolation recorded above, but all of these failed. Further inoculation



work was therefore postponed until the following spring, when more favorable conditions would obtain.

When the disease appeared in 1919, isolations were made from active young infections on leaves and petioles; and single colony subcultures from these were used for inoculating young vigorous cannas, obtained from a new source, in large pots in the hothouse. Suspensions from young agar cultures were sprayed into the youngest rolled leaf of some plants without wounding. In other cases the stalk below the lowest leaf blade was smeared with bacteria, and pricks were made through this to the young leaves within. Part of the inoculated plants of each lot were kept in cages and sprayed with sterile water for 36 hours; others were left in the open house. Controls in other cages were sprayed with sterile water.

Good prompt infections were obtained by both spray and prick inoculations on the plants that were kept in cages, and only fair infections on those in the open house that were pricked. Controls remained healthy. Infection was apparent on the sprayed plants only when the young, susceptible leaves which were tightly rolled at the time of inoculation emerged or unrolled, usually after six or seven days. On the oldest rolled leaves those spots which at this time showed as small stomatal infections did not progress further. On younger leaves the initial stomatal stage was past, the spots extending from vein to vein and beginning to lengthen into streaks (Pl. 36, A). On pricked plants kept in cages, infection was more rapid and destructive, as is shown on Plate 36, B, C. Here infection showed on the fourth day, running downward from pricks seen in the photograph near the tips of the leaves (X, X). These leaves were tightly rolled when pricked through the enveloping folds of older leaves. In the leaf shown on Plate 36, C, the streak from the pricks on the midrib was 3 cm. long on the fourth day. One day later it was 10 cm. long, and by the eleventh day it had reached almost to the base of the next older leaf, widening downward where inclosed by the sheathing petioles and killing the shoot completely. After the plants were once infected, secondary infections took place in some cases on young shoots which were in the same pots with inoculated shoots but which were too young at the time of inoculation to have been directly infected—that is, were without any leaf which had begun to unfold. Younger unfolding leaves on sprayed shoots also showed infection as they emerged some weeks later.

From several of these infections reisolutions were made, and inoculations with single colony transfers thus obtained gave typical infections on cannas when inoculated by spraying and by needle pricks. These isolations and reisolutions were used for cultural work in comparison with cultures of the previous year, with which they were found to agree.



## THE ORGANISM

## DESCRIPTION

The causal organism is a short rod with rounded ends, single, in pairs or chains, 1 to 2  $\mu$  long by 0.5 to 0.7  $\mu$  broad, when stained from 24-hour agar cultures. It is motile by means of one to three bipolar flagella (Pl. 38, B). It does not form spores, is Gram-negative, is not acid-fast and stains readily with the ordinary anilin stains. Capsules were stained from 10-day agar cultures with Ribbert's stain. Rods with swollen ends occur in old milk cultures.

CULTURAL CHARACTERS<sup>1</sup>

**AGAR PLATES.**—On + 15 (Fuller's scale) peptone-beef agar at 20° to 25° C., colonies appear on the second day. By the fourth day the surface colonies are 2 mm. in diameter, thin, white, round with entire margin, wet shining, finely granular, semitransparent, with internal concentric markings by oblique light, especially in the thinner margins. As colonies enlarge (5 to 8 mm.) they are white, slightly convex, and may be either round or irregularly scalloped (Pl. 37, C). The scallops are formed by wedges of more transparent growth in which distinct radiating lines are seen by direct transmitted light (Pl. 38, D). By oblique transmitted light the wedges show both radiating lines and also internal concentric markings (Pl. 38, E). In consistency they are viscid, becoming more so with age. Buried colonies are lenticular, becoming round to irregular.

**AGAR STABS.**—In agar stabs the surface growth is flat, wet shining, moderate, at first round, later with an undulate margin, then covering the entire surface. Stab growth is moderate, granular, tapering downward, ending at one-half the depth of the agar. In old cultures crystals form from the surface downward in ragged spears 1 to 2 cm. long. There is no discoloration of the agar.

**AGAR STREAKS.**—Two-day-old streaks from bouillon are filiform, 2 mm. wide, tapering upward, white, wet shining, with thin margins and granular center. Later (6 days old) growth is 4 to 5 mm. wide with finely scalloped edges and radiating lines by transmitted light, running from the granular center into the translucent margins. The V is half filled with white precipitate. The growth is very viscid.

**GELATIN PLATES.**—On gelatin plates kept at 15° C. colonies appear on the fourth day. At 20° they are visible on the second day. Growth is very slow and without liquefaction at 15°. Colonies are thin, round, later becoming flower-like—that is, with a crater-like center and wider scalloped margin (Pl. 38, C). At 21° to 24° very slow liquefaction occurs, beginning about the tenth day, especially on thickly sown plates. On thinly sown plates colonies usually lie in a shallow, dry saucer.

<sup>1</sup> Kahlbaum's agar and Difco peptone were used throughout.



**GELATIN STABS.**—There is slight granular growth along the entire line of puncture, best at the top where it is sometimes villous or papillate, the length of the villi or papillae decreasing downward. Surface growth when 6 weeks old at 15° to 18° C. is thin, white, transparent, slightly rugose, with an undulate margin. No liquefaction occurs within two months at this temperature, but at 21° to 25° a saucer of liquefaction 1 cm. deep may be formed within three weeks. Usually clusters of crystals form at the bottom of the liquefied part.

**WHEY AGAR<sup>1</sup> PLATES.**—Colonies on whey agar plates are white, round, convex, opalescent, with internal concentric markings by oblique light. On thickly sown plates by the third day, and on thinly sown plates by the fourth or fifth day, each colony is surrounded by a clear area 1 to 2 mm. wide, beyond which a white halo extends outward (Pl. 37, B). On thickly sown plates this involves the whole surface. As the colony grows it fills the clear space, even growing out into the white halo. The halo is composed of an alkaline precipitate, which is readily dissolved by acids.

**WHEY AGAR SLANTS.**—Streaked from beef bouillon, growth is moderate, white, filiform, 2 to 4 mm. wide with undulate margins. The whole surface of the agar becomes white-clouded except for a clear area 1 to 4 mm. wide closely surrounding the streak of growth (Pl. 37, D). This halo is dissolved by acids.

**POTATO CYLINDERS.**—Growth on steamed potato is scanty, spreading, dirty white, wet shining, transient, becoming pale brown. The potato is grayed. Diastasic action is feeble.

**BEEF BROTH.**—Peptonized beef broth (+15) clouds weakly within 24 hours at room temperatures (21° to 25° C.); often within this time it forms a heavier flocculent surface layer, which falls on the slightest agitation. In undisturbed cultures the clouding is often banded, the heavier bands at the top. A heavy, viscid pellicle forms, which often falls slowly, center first, the edges remaining attached to the walls, so that a hollow inverted cone is formed (Pl. 37, A), which lengthens slowly to reach from pellicle to precipitate, and may persist for weeks. This occurs in both alkaline and acid bouillons. The abundant viscid precipitate is granular, semitransparent, and does not form a compact mass. It rises in a tenaciously viscid swirl on shaking. Clouding becomes heavy and is persistent.

**MILK.**—Milk begins to clear on the fifth to the tenth day, and clearing is complete within four weeks. No coagulation takes place. The milk becomes golden brown on long standing, sometimes with a jelly-like consistency.

<sup>1</sup> Formula for whey agar: To 3 pints of milk heated to boiling add a 20 per cent solution of hydrochloric acid sufficient to coagulate the milk. Avoid excess of acid. Filter through cheesecloth. Add *N/4.5* sodium hydroxid until the whey titrates +7. To 500 cc. of whey add 150 cc. of water, 1.5 gm. of Nelson's photographic gelatin No. 1, 7.5 gm. of peptone, 7.5 gm. of agar flour, and 7.5 gm. of saccharose. Dissolve by steaming 20 minutes, clarify with white of egg, tube, and autoclave 15 minutes at 110° C.



**LITMUS MILK.**—Litmus milk blues rapidly and uniformly from the top downward, beginning on the second day. There is no coagulation, but a gradual uniform clearing. Reduction begins promptly, the color passing from blue to pale purplish gray (Ridgway,<sup>1</sup> *Pl. LIII*), drab gray (Ridgway, *Pl. XLVI*), light drab (Ridgway, *Pl. XLVI*), and tawny olive (Ridgway, *Pl. XXIX*). Later the blue color returns.

**METHYLENE BLUE MILK.**—Milk to which methylene blue was added to make it robin's egg blue shows reduction in color on the second day. In 10 days reduction is complete.

**COHN'S SOLUTION.**—Usually no clouding occurs in Cohn's solution. Occasionally very weak clouding takes place and a faint rim is formed. After six weeks in such cultures the fluid is clear with 3 to 6 mm. width of white precipitate which breaks up on shaking.

**USCHINSKY'S SOLUTION.**—In Uschinsky's solution clouding is heavy with a viscid pellicle falling like that in beef broth, and a heavy viscid precipitate.

**FERMENTATION TUBES.**—In fermentation tubes containing 1 per cent peptone plus 1 per cent saccharose, dextrose, lactose, maltose, glycerin, or mannit there is good clouding in the open end, but none in the closed end, and no gas. All give a slightly alkaline reaction to neutral litmus paper at the end of two weeks.

**LITMUS SUGAR AGAR.**—Litmus agar containing 1 per cent peptone water plus 1 per cent saccharose, dextrose, lactose, maltose, galactose, or glycerin is not reddened. Moderately good growth occurs on all.

**BLOOD SERUM.**—Growth on blood serum is moderate, with no liquefaction and no discoloration of the medium.

**CONGO RED AGAR.**—On congo red agar little or no growth occurs, but in a few cases it was sufficient to show that the red color is taken up by the organism.

**OPTIMUM REACTION FOR GROWTH IN BOUILLON.**—The organism grows best in +10 to +15 peptone-beef bouillon. It grows fairly well in +20 to +25 but not at all in +30. Growth is good in neutral bouillon and in -5 to -10, is weak in -15, very weak in -20, and no growth occurs in -25.

**TOLERATION OF ACIDS.**—Neutral peptone-beef bouillon was used, to which was added 0.1 per cent, 0.2 per cent, and 0.3 per cent of malic, tartaric, and citric acid, respectively. These titrated as follows:

Malic	0.1 per cent	+18	Tartaric	0.1 per cent	+23	Citric	0.1 per cent	+24
	.2 per cent	+32		.2 per cent	+37		.2 per cent	+40
	.3 per cent	+47		.3 per cent	+53		.3 per cent	+53

The organism grew readily in 0.1 per cent of all three acids, best in the malic, and weakest in the citric. No growth took place in 0.2 per cent or in 0.3 per cent of any of them. Other tests were made with

<sup>1</sup> RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.). Washington, D. C. 1912.



the following results: Growth in +32 malic acid, +37 citric acid, and +35 tartaric acid; no growth in +33 malic acid, +40 citric acid, and +37 tartaric acid.

**TOLERATION OF SODIUM CHLORID.**—Tests were made in +15 peptone bouillon to which were added 1, 2, 3, 4, and 5 per cent sodium chlorid. Prompt clouding, becoming heavy, appeared in 1 per cent, and fairly prompt moderate clouding appeared in the 2 per cent. The growth in 3 per cent was delayed and took the form of cobwebby, viscid, persistent, streamers without clouding. These streamers were not made up of chains but of single or paired organisms. No growth occurred in the presence of 4 or 5 per cent sodium chlorid.

**OTHER CULTURAL FEATURES.**—Growth is not retarded in bouillon over chloroform. Nitrates are strongly and promptly reduced. No indol is formed (20 days to 2 months). Hydrogen sulphid is produced in peptone-beef bouillon. Ammonia production is strong. The odor of most cultures is rather disagreeable.

**TEMPERATURE RELATIONS.**—The optimum temperature for growth is about 35° C. No growth takes place in peptone-beef bouillon (+15) below 5° nor above 40°. In one test there was very weak growth at 40°. The thermal death point is 52°.

**EFFECT OF FREEZING.**—When transfers from young +15 peptone bouillon cultures are frozen solid and kept frozen for 15 minutes, then thawed and plates poured with measured loops just as before freezing, the colony counts show that from 50 to 90 per cent are killed.

**EFFECT OF DESICCATION.**—The organism is very sensitive to drying. Drops of 1- to 6-day-old bouillon cultures were dried on cover glasses in sterile Petri dishes in the dark. These covers transferred to bouillon after 2 days' drying gave prompt clouding; after 3 days less than half gave growth, and after 5 days no growth was obtained.

**EFFECT OF SUNLIGHT.**—The organism is very sensitive to sunlight. Agar poured plates, one-half covered with black paper, were exposed to bright sunlight bottom side up on ice in November at 11.30 a. m. When counted 5 days later, colonies were numerous on the covered side. On the exposed side there was noticeable reduction after 1 minute's exposure; 75 per cent were killed after 2 minutes, 95 per cent after 3 minutes, and all were killed after 4½ minutes' exposure.

**VITALITY ON CULTURE MEDIA.**—The most long-continued growth is made in milk, peptone-beef bouillon, and peptone-beef agar. At room temperatures the organism will live in these media for 6 or 7 months, or until the medium is almost completely evaporated. Cultures in these media kept in the ice box for 1 year give prompt growth when transferred.



## GROUP NUMBER

According to the chart of the Society of American Bacteriologists<sup>1</sup> the group number of this organism is 211.3333023.

## TECHNICAL DESCRIPTION

***Bacterium cannae*, n. sp.**

A short rod with rounded ends; chains; flagella 1 to 3, bi-polar; capsules; no pseudo-zoogloae; aerobic; nonchromogenic; liquefies gelatin very slowly; diastasic action weak; reduces nitrates; does not produce acid or gas from sugars; clears milk; blues, then reduces litmus milk without coagulation; does not produce indol; produces hydrogen sulphid and ammonia; grows in Fermi's and Uschinsky's solutions and very feebly or not at all in Cohn's solution; optimum temperature 35° C., maximum 40°, minimum 5°; thermal death point 52°; vitality at room temperatures on media six months; Gram-negative, not acid-fast; sensitive to drying; moderately tolerant of acids and alkalis; sensitive to freezing and to sunlight. The cause of a meristematic disease cultivated in cannas.

## SUMMARY

The budrot of cannas is a hitherto undescribed bacterial disease caused by *Bacterium cannae*, n. sp. The disease is primarily one of young tissues and moist conditions.

Infection takes place through the stomata and spreads through the intercellular spaces of the parenchyma of leaf blade, petiole, and stalk.

It is most destructive early in the season, that is on young plants. It begins in the hothouse and continues in the open beds. It destroys the buds, forms large unsightly spots on the leaves, and ruins the blossom clusters by blighting the flower buds or by decaying the stalk.

The method of overwintering whether in the soil or on the rootstocks, or both, is uncertain as yet. Although no means of control has been worked out, it is recommended as a preventive measure that rootstocks for planting be selected as far as possible from healthy stock only, that care be observed to avoid crowding and overwatering before setting out, that good ventilation be maintained in the houses, and that specially sensitive varieties be discarded.

<sup>1</sup> SOCIETY OF AMERICAN BACTERIOLOGISTS. DESCRIPTIVE CHART. Indorsed by the society for general use at the annual meeting Dec. 31, 1914. Prepared by the committee on revision of chart identification of bacterial species.







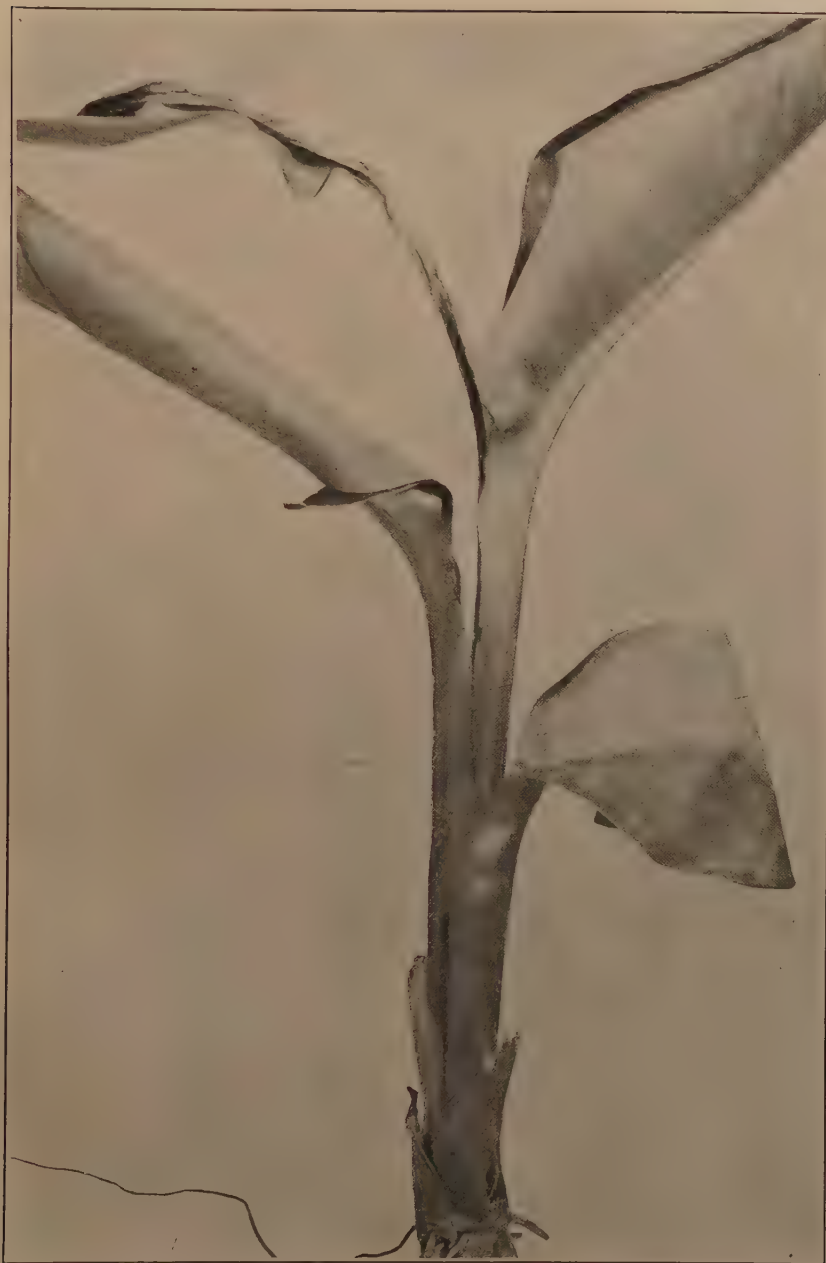
PLATE 31<sup>1</sup>

Young canna shoot (natural infection) in which the bud has been killed. *Bacterium cannae* was plated from the interior 1 inch from the base of the shoot. Root-stock healthy.

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<sup>1</sup> All photographs in Plates 31 to 38 are by Mr. James F. Brewer.







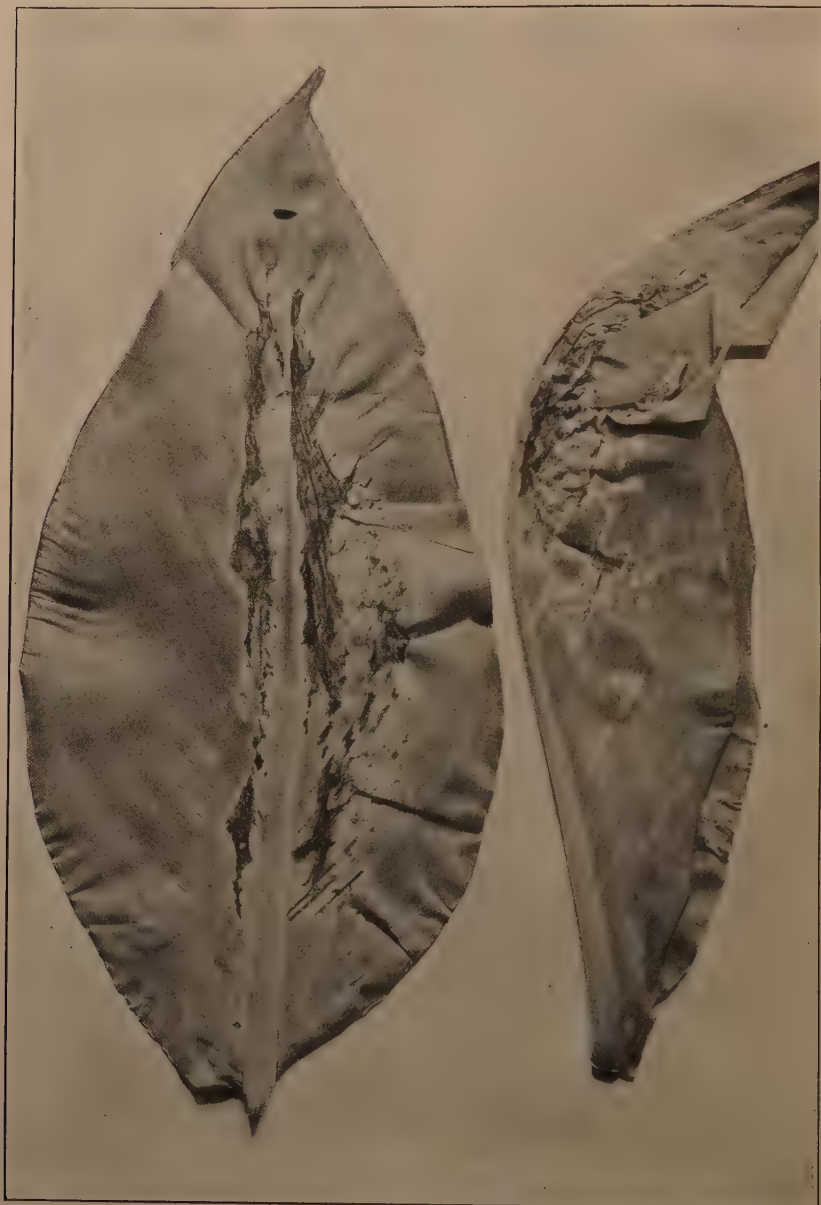




PLATE 32

Canna leaves (natural infection), showing character of spots and distortion of leaves. These were put under glass to straighten sufficiently for a photograph. Note lighter (yellowed) areas and minute stomatal infections in the vicinity of large spots. Much reduced.

PLATE 33

Canna leaves (natural infection), showing disease running down the petioles from large leaf spots. Compare Plate 36, A, where the infection is just reaching the midrib.





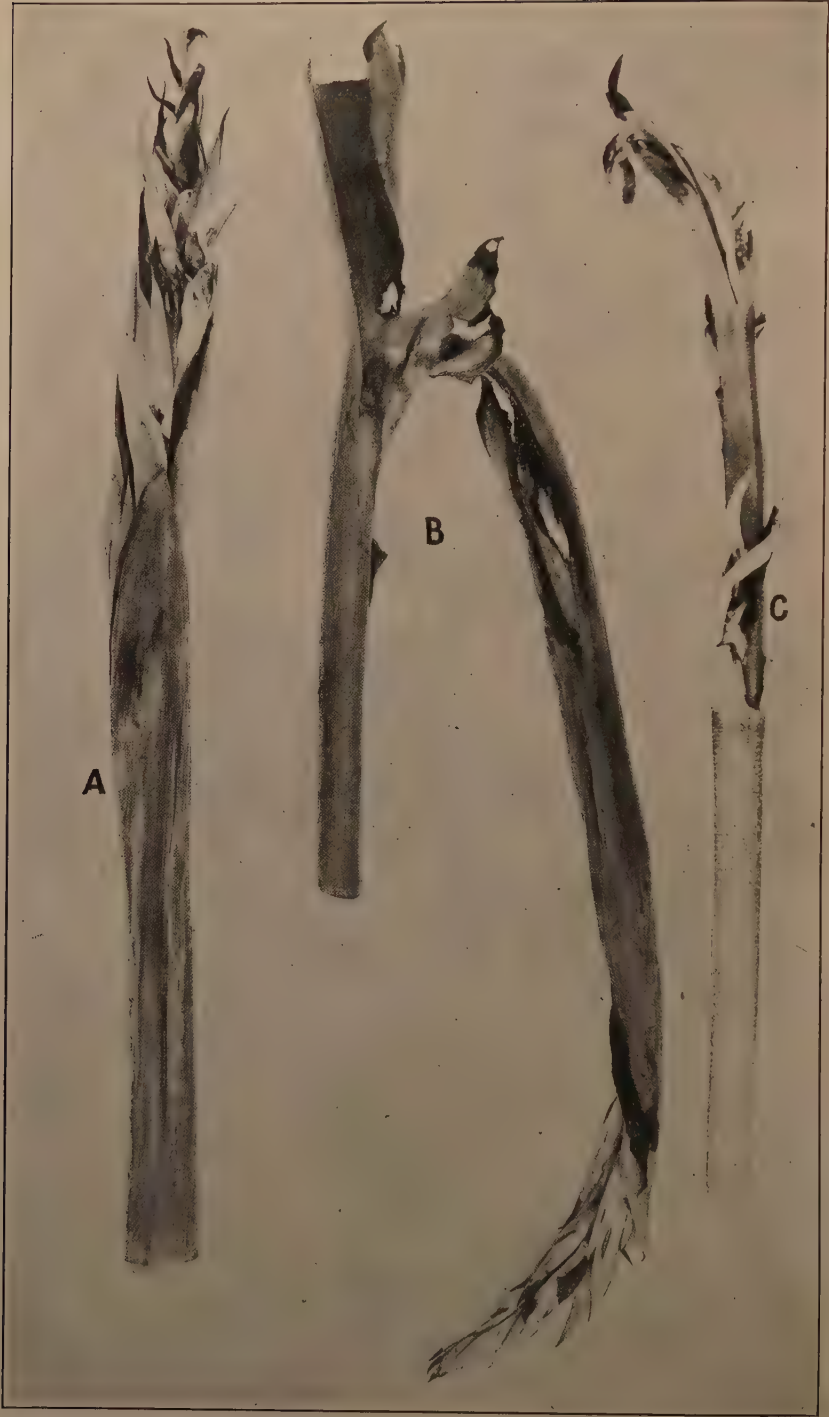




PLATE 34

Blossom clusters (natural infection).

A, C.—Infection on the blossom buds (blackened) of uninjured stalks.

B.—Stalk decayed on one side and broken over while the buds are only slightly infected.

PLATE 35

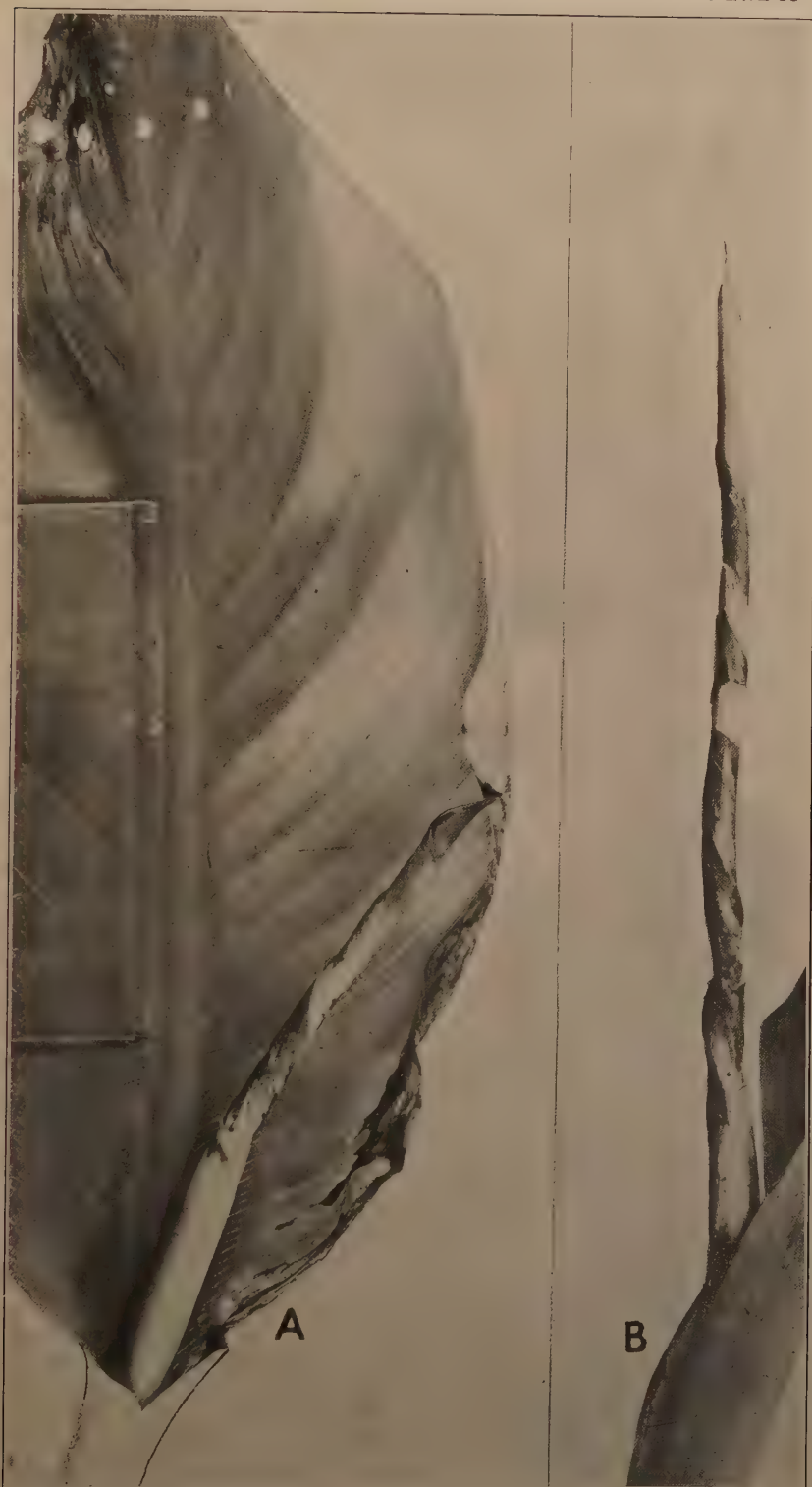
Tightly rolled buds showing infection.

A.—Bud moderately infected, and next older leaf with infections at base and at tip.

B.—Badly infected bud. Entire blackened area diseased.

Natural infections.





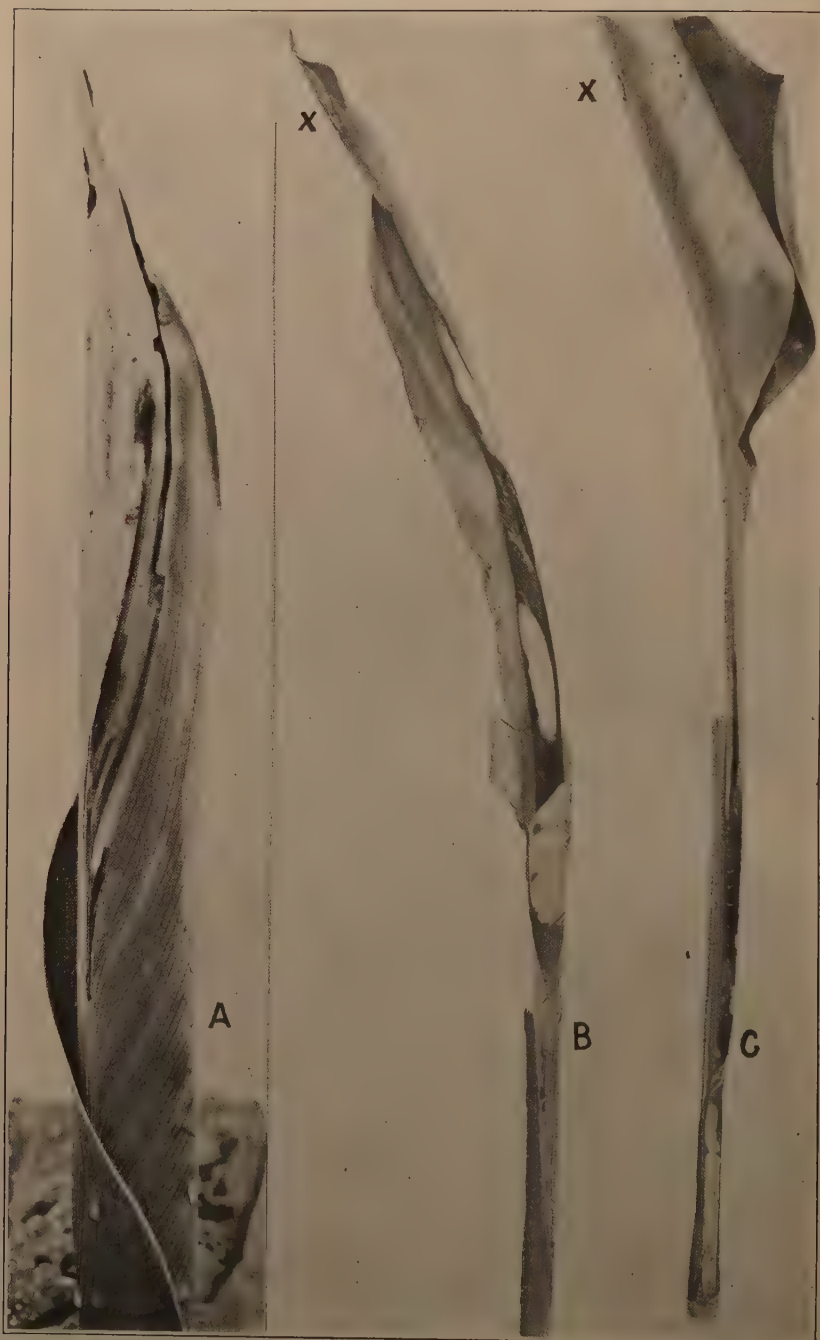




PLATE 36

Results of pure culture inoculations.

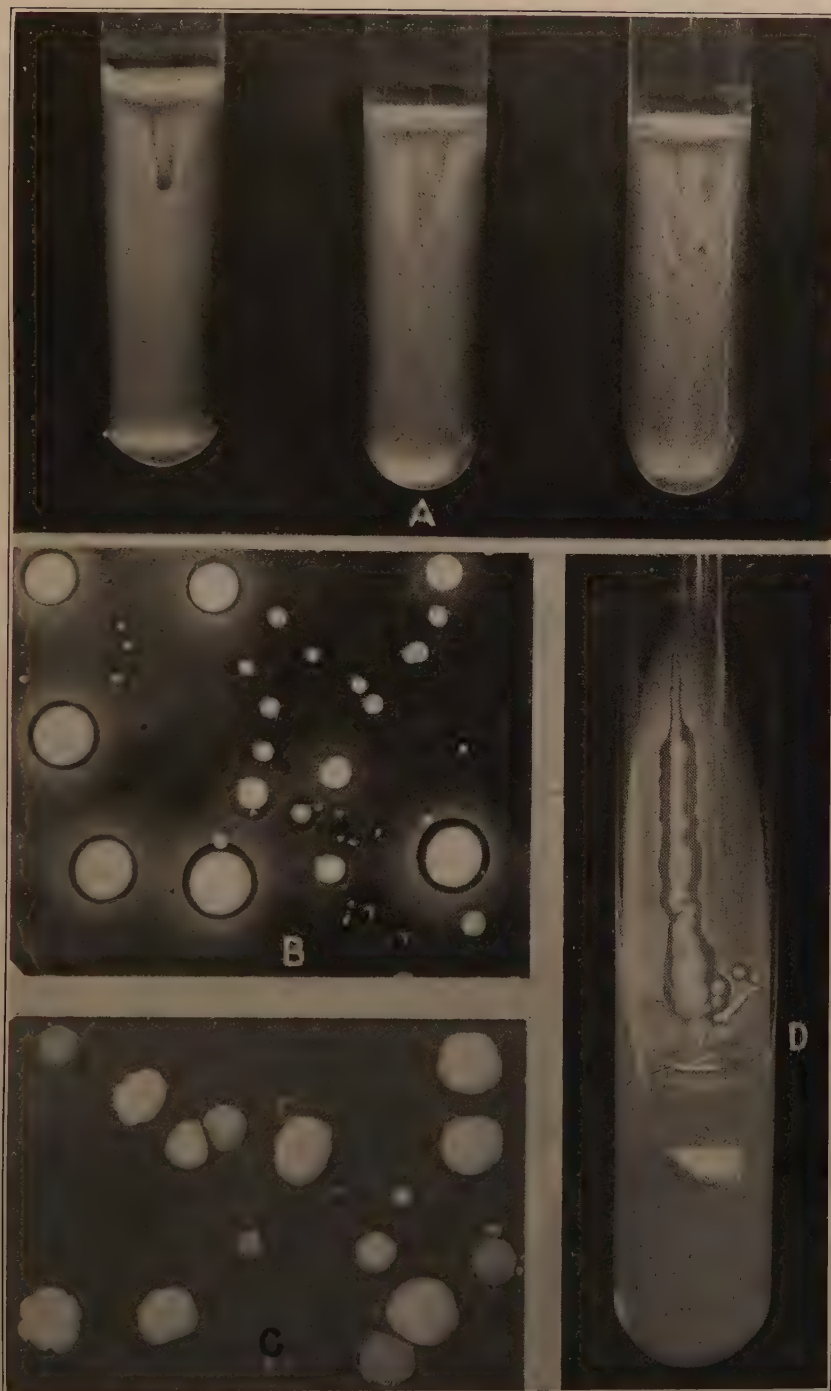
A.—Second infected leaf on a very young shoot inoculated by spraying, 18 days after inoculation. In 5 days infection ran from water-soaked spots near the tip into the midrib as shown in the figure.

B, C.—Needle prick inoculations 11 days old. Infection has run down from pricks in upper part of each. Blackened areas are diseased. Part of leaf cut away in both to show decay within. In C observe cracks in the blackened area caused by the stretching of the sound side.

PLATE 37

- A.—Cultures in + 10 beef bouillon, 8 days old, showing viscid, persistent, falling pellicle in different stages.
- B.—Colonies on 8-day-old whey agar plates.  $\times 2$ .
- C.—Colonies on beef agar 11 days old, showing shape.  $\times 2$ .
- D.—Whey agar slant 3 days old, showing clear area and alkaline halo.





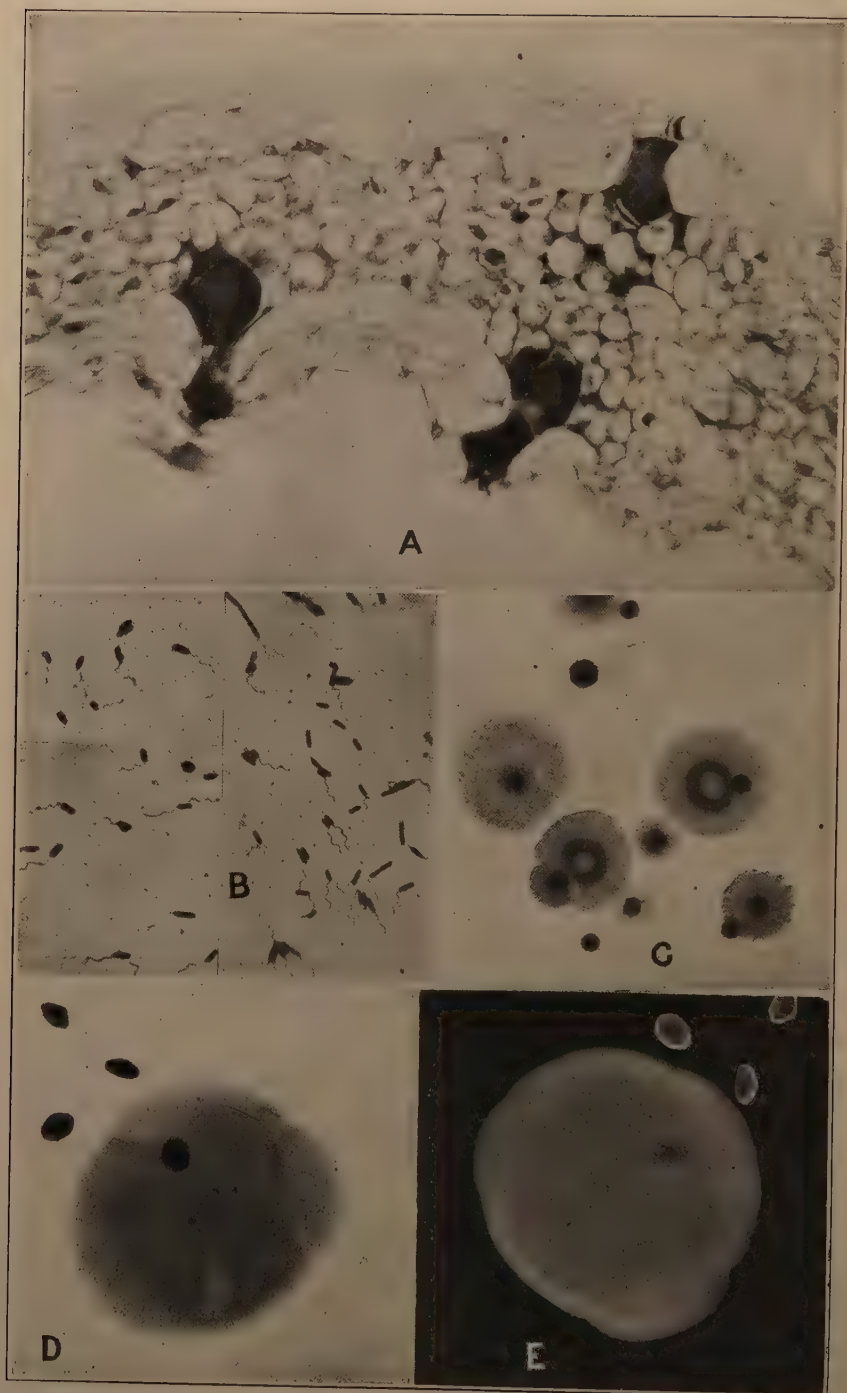


PLATE 38

A.—Section of young leaf with stomatal infections. Bacteria have penetrated the intercellular spaces to the center of the leaf from the upper infection at the right. The cloud below the lower left infection is composed of bacteria. Stained with Ziehl's carbol fuchsin.

B.—*Bacterium cannae*, showing flagella.  $\times 850$ .

C.—Colonies on gelatin plate 7 days old at  $15^{\circ}$  to  $18^{\circ}$  C.  $\times 10$ .

D.—Agar plate colony by direct transmitted light, showing radiating lines and 3 buried colonies.  $\times 10$ .

E.—Same colony as in D but by oblique transmitted light, showing internal concentric markings and radiating lines.  $\times 10$ .











